REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-8, 11-19, 22-29, 32-39, 42-61 and 84-91 and 7-10 are in this case. Claims 48- 61 have been withdrawn from consideration. Claims 1-7, 12-18, 22-28, 32-38, 42-47 and 84-91 has been rejected. Claims 8, 11, 19, 29 and 39 have been objected to. No Claims have been allowed. Claims 14-19, 22-23 30-33, 40- 41, 87 and 91 have now been canceled, rendering moot the Examiner's rejections thereof. Claims 1, 12, 24, 34, 84-86 and 90 have now been amended.

35 U.S.C. §112, First Paragraph, Rejections

The Examiner has rejected claims 1-7, 12-18, 22-28, 32-38, 42-47 and 84-911-7, 12-18, 22-28, 32-38, 42-47 and 84-91 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one of ordinary skill in the art to make and/or use the invention. The Examiners rejections are respectfully traversed. Claims 14-19, 22-23 30-33, 40- 41, 87 and 91 have now been cancelled, rendering moot the Examiner's rejection thereof. Claims 1, 12, 24, 34, 84-86 and 90 has now been amended.

The Examiner has stated that while the specification clearly discloses the successful isolation of a novel flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase gene from Pummelo using degenerate PCR primers designed from fragments of the digested enzyme, the instant specification does not describe any other sequences that would encode a polypeptide having flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase activity, ... or which sequences of the peptide are conserved or known motifs and would allow for isolation of functional equivalents. Thus, the Examiner has asserted that the instant specification does not describe the genus comprising functional embodiments that fall within the range of the invention as claimed.

Applicant wishes to reemphasize that the present invention is of a novel, unique 1-2-rhamnosyl-transferase gene that was isolated and cloned from Pummelo young leaf mRNA by RT-PCR using unique gene specific PCR, and the recombinant protein product thereof (SEQ ID NO: 21). Although the glucosyl transferase family is large, and several glucosyl transferases catalyzing the transfer of UDP-sugar to

flavonoids have been described, the specific flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase (α-1-2 rhamnosyl transferase) activity of the polypeptide encoded by the polynucleotides of the present invention had not been demonstrated for any other known sequences at the time the application was filed.

As stated in our previous communication, dated June 3, 2003, as the coding sequence of the 1-2-rhamnosyl-transferase gene of the present invention has been proven to be unique, one of ordinary skill in the art, in possession of the teachings of the present invention, would have a reasonably high expectation of identifying and isolating all polynucleotides having both high sequence homology to SEQ ID NO: 20, and encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity using techniques of hybridization and enzyme assay as described in the instant specification or, for example, in *Short Protocols in Molecular Biology,* Second Edition, Ausabel et al. ed, John Wiley and Sons, 1992.

Indeed, the inventors have very recently reported the discovery of a previously unknown sequence having a high degree of homology to the Pummelo flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase gene coding sequence disclosed in the instant specification (SEQ ID NO:20). Employing the teachings and protocols of the present invention, Applicant performed a homology search and identified a recently published EST from a *Poincirus trifoliata* (Japanese- or bitter orange) cDNA library (CF 419914), having a > 90 % sequence identity with a large portion of the Pummelo flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase gene sequence (SEQ ID NO:20) of the present invention (see Appendices I and II, enclosed herein, and accompanying the Declaration cofiled herewith). The translated amino acid sequence of the *P. trifoliata* EST also displays a high (> 90 %) identity to the amino acid sequence of the Pummelo flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase polypeptide of the present invention (SEQ ID NO:21) (see amino acid alignment, Appendix III, enclosed herein, and accompanying the Declaration). Yet further, it will be noted that the translation product of the *P. trifoliata* EST, exhibits only very low homology (< 50%) to any sequences other than SEQ ID NO:21, and shows some limited homology to polypeptide domains common to gluronyl-transferases, indicating a

functional homology as well as structural homology to the flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase enzyme of the present invention. Thus, it is Applicant's strong opinion that the instant specification provides an adequate written description of the function and structural features common to polynucleotides having a high degree of homology in SEQ ID NO: 20, encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase.

That notwithstanding, and to further clarify and define the metes and bounds of the present invention, and to expedite prosecution in this case, as recommended by the Examiner during the abovementioned telephone interview of October 7, 2003, and in communications thereafter, currently amended independent claims 1, 24, 34, 84 and 88 have now been amended to recite the limitations of:

> "...a nucleic acid sequence encoding a polypeptide having at least 95 % sequence identity with SEQ ID NO: 21 ..."

Further, as recommended by the Examiner, all reference to homology as determined by hybridization under conditions of high stringency has been removed from the claims. Thus, the now amended independent claims, and all claims directly or indirectly depending therefrom, relate to all polynucleotides encoding polypeptides having high sequence homology (at least 95 %) to SEQ ID NO: 21 and having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity.

Support for such an amendment can be found throughout the instant specification, for example, page 9, lines 25-34:

> "According to another embodiment of the present invention, the nucleotide sequence shares between...90 and 100% identical bases with SEQ ID NO:20",

page 10, lines 12-19:

> "...the polypeptide encoded by the nucleotide sequence of the present invention can share between...90 and 100% identical

or conserved amino acids with SEQ ID NO:21 or a functional part thereof..."

and page 12, lines 6-10:

"Homology may be at the nucleotide sequence or amino acid sequence level. Preferably, the nucleic acid or amino acid sequence of the homolog...shares homology with SEQ ID NOs:20 or 21, respectively, ...most preferably at least 90% homology..."

Applicant wishes to point out that the "at least 95% homology" of nucleic acid or amino acid sequences recited in the now amended claims clearly falls within the ranges of "between 90 and 100%" and "at least 90%" identity, as disclosed in the instant specification.

The Examiner has further asserted that methods directed towards using antisense, using polynucleotides having 80% sequence identity for modifying gene expression in a plant, are unpredictable, because of the requirement for specific annealing, and that the use of sequences falling within the range of at least 80% sequence identity would require undue experimentation to eliminate non-functional embodiments.

As described hereinabove, now amended independent claim 24 recites:

"...wherein the transgenic plant is genetically modified to include an expressible polynucleotide comprising a nucleic acid sequence having at least 95% sequence identity with SEQ ID NO:20...said polynucleotide designed encoding nucleotide sequences complementary to, and capable of binding to flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase transcripts..."

Thus, now amended claim 24, and claims depending therefrom, relate to antisense methods using polynucleotides having at least 95% sequence identity with SEQ ID NO:20, according to Examiner's recommendations. Using such polynucleotides having at least 95% sequence identity for modifying gene expression in a plant allows specific annealing of the sense and antisense polynucleotides under physiological conditions (see, for example, *Short Protocols in Molecular Biology,* Second Edition, Ausabel et al. ed, John Wiley and Sons, 1992), and is thus sufficiently predictable, and would not require undue experimentation to eliminate non-functional embodiments. Support for such amendments is found throughout the instant specification, as detailed hereinabove.

Regarding claim 84, the Examiner has noted that said claim is not limited to any particular means of "regulating" gene expression. Applicant wishes to point out that currently amended claim 84 now recites the limitation of "regulating the level of activity or expression of a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase mRNA in (a) plant cell":

> "A method of modifying a level of a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase activity in a plant cell, the method comprising regulating the level of activity or expression of a 7-*O*-glucoside-2"-*O*-rhamnosyl-transferase mRNA in the plant cell, said 7-*O*-glucoside-2"-*O*-rhamnosyl-transferase mRNA comprising a polynucleotide sequence being at least 95% complementary to SEQ ID NO: 20 as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 4, thereby modifying the level of a 7-*O*-glucoside-2"-*O*-rhamnosyl-transferase activity in the plant cell."

as well as the limitation of the "7-*O*-glucoside-2"-*O*-rhamnosyl-transferase mRNA comprising a polynucleotide sequence being at least 95% complementary to SEQ ID NO: 20". Thus, currently amended independent claim 84 now reads on

methods of modifying 7-*O*-glucoside-2"-*O*-rhamnosyl-transferase activity by regulating levels of specific 7-*O*-glucoside-2"-*O*-rhamnosyl-transferase mRNA.

The Examiner has further noted that currently pending claims 85 and 87 do not recite any degree of sequence identity. Applicant wishes to point out that currently amended claim 85, and claim 87 dependent therefrom, now recite the limitation of "an exogenous polynucleotide encoding a polypeptide having at least 95% sequence identity with SEQ ID NO:21..." and "a nucleic acid sequence as set forth in SEQ ID NO:20...", according to the Examiner's recommendations.

Thus, the instant specification provides one of ordinary skill in the art with generous guidance as to how to practice the method of the instant invention as disclosed in currently amended independent claims 1, 24, 34, 84, and 88, and claims dependent therefrom.

In view of the above arguments and amendments, Applicant believes to have overcome the 35 U.S.C. § 112, first paragraph rejections.

Claims Objections

The Examiner has objected to claims 8, 11, 19, 29 and 39 as being dependent upon rejected base claims. Claim 19 has now been canceled, rendering moot the Examiner's objection thereto. Applicant wishes to point out that pending independent claims 1, 24 and 34, from which dependent claims 8 and 11, 29, and 39, respectively, derive antecedent basis, have now been amended to include the limitations of

> "...a nucleic acid sequence encoding a polypeptide having at least 95 % sequence identity with SEQ ID NO: 21...",

as recommended by the Examiner, overcoming rejections thereof based on 35 U.S.C. § 112, first paragraph. Thus, the Examiner's objections to claims 8, 11, 29 and 39 are traversed by virtue of currently amended base claims 1, 24, and 34 now being in condition for allowance.

In view of the above amendments and remarks it is respectfully submitted that claims 1-8, 11-13, 24-29, 34-39, 42-47, 84-86, and 88-90 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

An early and favorable action is therefore respectfully requested.

Respectfully submitted,

Sol Sheinbein
Registration No. 25,457

Date: November 19, 2003.

Encl.
Declaration by Dr. Yoram Eyal; and
Appndicers I - IV